Environmental Health Perspectives
Vol. 51, pp. 67-72, 1983

Macrophage Functions after Exposure to Mineral Fibers

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UICC, other well-defined asbestos samples and different man-made mineral fibers (MMM) such as glass fiber and synthetic amphibole asbestos were studied *in vitro* by using rat and guinea pig lung macrophages. These samples had relatively narrow length and diameter spectra.

Most of the fiber samples were added to the cultures on a gravimetric basis, although some were added on a numerical basis. Electrocorundum and DQ_{12} (Dorentruper Quartz) were used as controls at comparable gravimetrical concentrations.

The assays used were the release of lactate dehydrogenase (to demonstrate plasma membrane permeability) and the release of β-glucuronidase (to indicate lysosomal permeability). Carbohydrate metabolism was monitored by the measurement of lactic acid production and, as one of the tests for macrophage function, the production of lysozyme was determined.

The phagocytic ability of the cells was measured, after the addition of opsonized zymosan, by bioluminescence following luminol enhancement. Only some results could be evaluated, however, due to technical difficulties.

A length- and dose-dependent cytotoxicity of the fibers was found in this system which was similar to that previously described with permanent cell lines. No great differences were found between fibers having different physicochemical compositions if their geometric dimensions were similar. Long, very thin fibers of glass, chrysotile, crocidolite and synthetic fluoroamphiboles were all toxic in the test system.

Introduction

The macrophage plays a central role in the manifestation of inflammation (*1*). Furthermore, macrophages are the first target cells with which environmental and occupational dusts come into contact after they have been inhaled into the lung (*2*).

Silica, and asbestos fibers, have been recognized as being toxic in *in vitro* macrophage cell systems and this cytotoxicity is characterized by the release of cytoplasmic and lysosomal enzymes (*3*, *4*). However, Davies et al. (*5*) have demonstrated that peritoneal macrophages can also release acid lysosomal hydrolases in the absence of cytoplasmic enzyme secretion, after phagocytosis of chrysotile.

It has been demonstrated that macrophages respond differently to different fibers and doses and that the response is also dependent on the animal species used (*6-8*). However, it is known that the macrophage is an immunocompetent cell which performs a number of functions connected with the defense mechanisms of the lung. Although these immunological functions have only been poorly examined after fiber exposures (*9*), the production of lysozyme and a phagocytic ability are known to be among its capabilities.

The geometrical dimensions of fibers seem to be an important factor in the pathogenesis of both fibrosis and cancer.

Materials and Methods

Cell Cultures of Alveolar Macrophages

Unstimulated guinea pig and rat lung macrophages were obtained by pulmonary lavage using heparinized (8 U/mL) calcium- and magnesium-free phosphate-buffered saline (PBS) (*10*, *11*). The resulting cell suspension was washed three times in heparin-free PBS, centrifuged at 350*g*, resuspended in serum-free Minimal Essential Medium (MEM) and adjusted to 1×10^6 cells/mL. Rat lung macrophages, 0.1×10^6, were then transferred to the cavities of microtiter plates (Falcon Plastics, No. 3040F) and 0.75×10^6 guinea pig cells were transferred to the cavities of multiwell plates (Falcon Plastics, No. 3008).

After 2 hr incubation at 37°C in a 5% CO_2/95%

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air mixture to allow cell attachment, the MEM was removed and different doses of the dusts were added in McCoy's 5A medium in the presence or absence of heat inactivated fetal calf serum.

For phagocytic determination, the cells were cultured in special glass vessels at a concentration of 0.2×10^6 cells/tube, but the incubation conditions were the same as those given above.

Fibers and Control Dusts

Fiber fractions from different sources were used. Some of them were prepared by using the technique of Spurny et al. (*12*) as previously described (*13*). The other fibers were a gift of The Johns Manville Corp (U.S.A.), and their physical characteristics are shown in Table 1.

Table 1. Length and diameter distributions of fibers.

		Length distribution		Diameter distribution	
No.	Fiber type	Length, μm	% of fibers	Diameter, μm	%of fibers
1	Short, very thin	<1	83.8	<0.1	52.0
	glass fibers	1.0-1.9	11.5	0.1-0.14	35.4
	(4106-23-7)	>1.9	4.8	0.15-0.19	5.7
				0.2-0.29	6.9
2	Long, very thin	1.0-2.9	9.2	<0.1	63.7
	glass fibers	3.0-4.9	15.8	0.1-0.14	25.6
	(4106-24-2)	5.0-9.9	25.0	0.15-0.19	8.8
		10-19	30.0	0.2-0.29	1.6
		20-29	9.2	0.3-0.59	0.3
		30-59	10.8	(0.41)	
3	Short, thin	1.0-1.9	14.1	<0.15	5.9
	glass fibers	2.0-2.9	35.2	0.15-0.29	16.4
	(4106-4-3)	3.0-4.9	35.2	0.3-0.59	35.4
		5.0-9.9	8.5	0.6-0.99	26.2
		10-22	7.0	1.0-1.99	16.1
4	Long, thin	<5	0.7	<0.15	51.4
	glass fibers	5.9-9	7.0	0.15-0.19	19.6
	(4106-19-2)	10-19	37.5	0.20-0.29	11.9
		20-39	36.0	0.30-0.39	6.1
		40-59	14.8	0.40-0.59	6.2
		60-99	4.0	0.60-1.49	4.8
5	Short, thick	1.0-2.9	22.4	0.4-0.59	3.4
	glass fibers	3.0-4.9	33.5	0.6-0.99	5.8
	(4106-6-1)	5.0-9.9	31.5	1.00-1.49	15.2
		10-19	10.5	1.50-1.99	28.6
		20-29	2.1	3.00-3.95	17.6
6	Long, thick	3.0-4.9	9.9	0.2-0.59	4.1
	glass fibers	5.0-9.9	14.6	0.6-0.99	18.0
	(4106-19-1a)	10-19	28.9	1.0-1.49	35.2
		20-29	20.1	1.5-1.99	22.7
		30-59	22.1	2.0-2.95	14.8
		60-99	4.4	3.0-5.95	5.2
7	Jeffrey fibrils	<10	29.5	0.03-0.05	99.9
	(chrysotile)	1.0-1.9	34.7		
	(4173-46-1)	2.0-4.9	27.6		
		5.0-9.9	6.6		
		10-19	1.6		
8	Jeffrey fibrils	0.10-0.99	11.8	0.10	99.9
	(chrysotile)	1.0-1.9	20.0		
	(4173-46-2)	2.0-4.9	24.1		
		5.0-9.9	20.5		
		10-19	17.7		
		20-39	5.9		
9	Short Munroe fibrils	0.40-9.99	22.5	0.03-0.05	99.9
	(chrysotile)	1.0-1.49	29.7		
	(4173-60-1)	1.5-1.99	21.0		
		2.0-2.99	15.3		
		3.0-4.99	10.0		
		5.0-9.99	1.7		

Table 1. (Continued).

No.	Fiber type	Length distribution		Diameter distribution	
		Length, μm	% of fibers	Diameter, μm	% of fibers
10	Long Munroe chrysotile (4173-60-2)	<3.0	0.0	<0.1	40.2
		3.0-4.9	0.7	0.1-0.29	20.2
		5.0-9.9	4.3	0.3-0.59	8.8
		10-19	26.4	0.6-0.99	13.6
		20-60	65.7	1.0-1.99	4.2
		60-80	2.9	2.0-2.5	13.3
11	Short crocidolite (S. Africa)	0.40-0.99	17.6	0.03-0.04	1.2
		1.0-1.9	44.4	0.05-0.09	7.5
		2.0-4.9	19.8	0.10-0.19	43.3
		5.0-9.9	6.2	0.20-0.39	32.4
		10-19	1.6	0.40-0.59	4.4
		20-29	0.4	0.60-0.99	1.2
12	Short crocidolite (4106-23-9)	0.1-0.69	47.8	0.05-0.09	25.3
		0.7-0.99	22.3	0.10-0.14	40.0
		1.0-2.9	24.6	0.15-0.19	19.4
		3.0-4.9	3.5	0.20-0.29	13.3
		5.0-9.9	1.5	0.30-0.39	2.0
		10-19	0.3		
13	Long crocidolite (4106-22-4)	3.0-4.9	4.2	0.10-0.14	27.8
		5.0-9.9	13.6	0.15-0.19	15.0
		10-19	28.3	0.20-0.29	30.9
		20-29	23.0	0.30-0.39	13.1
		30-59	24.1	0.4-1.49	7.9
		60-129	6.8		
14	Short fluoroamphibole (4106-31-3)	1	89.9	0.03-0.09	46.0
		1.0-1.9	5.8	0.10-0.19	40.9
		2.0-4.9	3.6	0.20-0.39	9.6
		5.0-19.0	0.75	0.40-0.99	2.9
				1.0-2.95	0.6
15	Respirable fluoroamphibole (4106-30-1)	0.5-1.49	8.2	0.03-0.04	44.7
		1.5-2.9	21.5	0.05-0.14	25.3
		3.0-4.9	27.0	0.15-0.39	20.9
		5.0-9.9	27.5	0.40-0.99	5.6
		10-19	12.5	1.0-2.0	3.5
		20-39	3.5		

The samples prepared by using the technique of Spurny et al. (*12*) have been defined by length, but those from The Johns Manville Corp. have also been defined by diameter.

Dörentruper quartz (DQ_{12}) and electrocorundum with a similar size distribution served as control dusts.

All of the dust samples were weighed and sterilized by dry heat before being suspended in medium by the use of ultrasonics.

Assay Systems

Lactate dehydrogenase (LDH) and lactic acid were estimated by using biochemical test kits (Boehringer GmbH, Mannheim, FRG). β-Glucuronidase was also measured by using a test kit (Sigma Chemie GmbH, München, FRG) and the lysozyme concentration was measured by using reagents supplied by Behringwerke AG (Marburg, FRG).

Phagocytosis was assayed quantitatively by determining the amount of luminescence produced after the addition of serum-opsonized zymosan A particles (Sigma Chemie GmbH, München, FRG). The chemiluminescence was measured after luminol (Lumac, Düsseldorf, FRG) enhancement in a six-channel apparatus (Biolumat 9505; Berthold, Wildbad, FRG). The results were printed out in counts/minute (cpm) using a teletype.

Results and Discussion

Modified UICC samples, well-defined fiber fractions and also glass fibers (*12*) cause release of the cytoplasmic enzyme LDH and the lysosomal enzyme β-glucuronidase from guinea pig alveolar macrophages after incubation with the relatively low concentration of 100 fibers/cell (Table 2). At this concentration, we do not find a significant difference between the two parameters. Table 2 also shows that the cytotoxicity is dependent on fiber length for all four fiber types. It is interesting to note that

Table 2. Enzyme release of guinea pig lung macrophages 24 hr after incubation with asbestos and glass fiber fractions of three well-defined length spectra.[a]

Expt. no.		Enzyme release, mU/mL	
		LDH	β-Glucuronidase
1	K_o	31	58
	K_{rd}	35	53
	K_F	112	98
	K_{FF}	68	101
	K_{fF}	47	45
2	K_o	30	52
	K_{rd}	31	49
	A_F	92	90
	A_{FF}	60	75
	A_{fF}	61	74
3	K_o	23	33
	K_{rd}	26	35
	C_f	51	83
	C_{FF}	44	57
	C_{fF}	41	48
4	K_o	28	37
	K_{rd}	31	34
	$G_{F_{100}}$	88	74
	G_{FF}	70	61
	G_{fF}	42	40

[a] Dust concentrations = 100 fibers/cell.

chrysotile showed a relatively low toxicity in comparison with crocidolite, amosite and glass fiber. This may be due to the substantial gravimetric differences that occur when the same numerical dose is used. For example, there would be a 2-fold weight increase for crocidolite and a 10-fold increase for amosite and glass fiber when compared to the weight of chrysotile.

These findings are in agreement with the results of Davies (*8*) and demonstrate that, at low fiber concentrations, any differential release of β-glucuronidase relative to LDH is low in contrast to that seen at higher concentrations (*6*, *7*).

Figures 1-3 show the results obtained with 15 new fiber fractions of glass fiber, chrysotile, crocidolite and synthetic fluoroamphiboles, all of which had interesting geometric dimensions. Figure 1 shows the amount of LDH released into the medium in the presence of serum after an incubation time of 20 hr. The results are expressed as a percentage with the cell control equaling 0% and quartz DQ_{12} equaling 100%. Figure 2 gives the equivalent results obtained when serum was absent and Figure 3 shows the lactic acid production, after a similar incubation period in the absence of serum, and the results are expressed as a percentage of the lactic acid produced by the control cells. With the exception of the synthetic amphibole, LDH release was dependent on fiber length in the presence or absence of serum in the medium.

A comparison of the three pairs of glass fiber

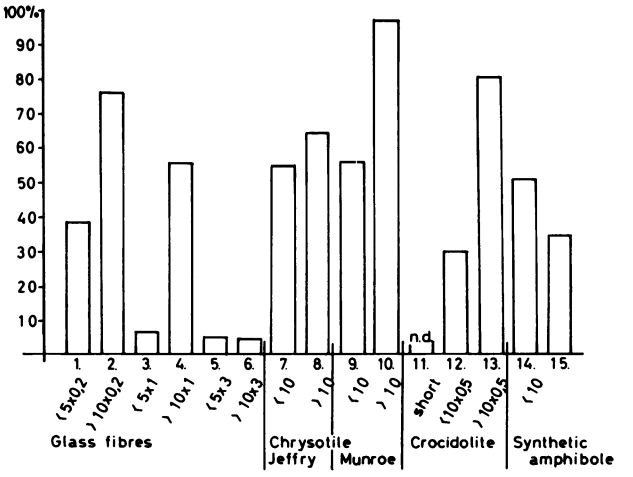

FIGURE 1. LDH release of guinea pig lung macrophages 20 hr after incubation with 100 μg dust/10^6 cells. Control = 0%; DQ_{12} = 100 %.

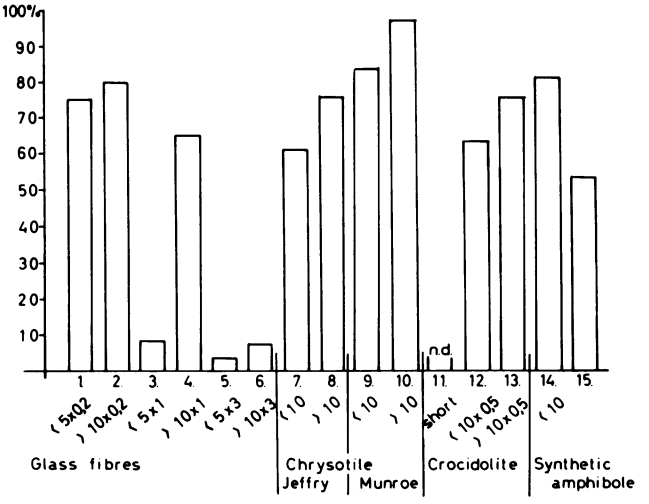

FIGURE 2. LDH release of guinea pig lung macrophages 20 hr after incubation with 100 μg dust/10^6 cells. Control = 0%; DQ_{12} = 100%.

samples shows that fiber toxicity is clearly dependent on both diameter and length. As no data were available on the number of fibers per unit weight, it was not possible to do further tests with different numerical concentrations. In contrast to our earlier investigations (*13*), it can be seen that even every short fiber can exhibit a high toxicity. The important difference between this study and the earlier tests lies in the very thin fiber diameter (thinner than 0.03 μm) in this study, in comparison to a mean diameter of 0.19 μm in the preparation made by Spurny et al. (*12*). In fiber fractions with a diameter of 1.0 μm, only the longer fibers cause a high release of LDH. Short fibers of this diameter and both fiber

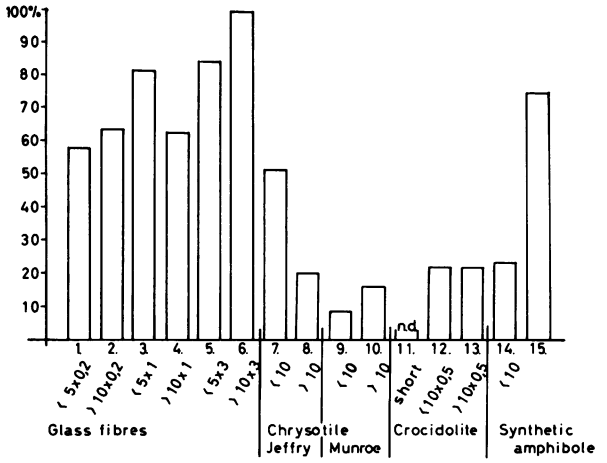

FIGURE 3. Lactic acid production of guinea pig lung macrophages 20 hr after incubation with 100 μg dust/10^6 cells. Control = 100%; DQ_{12} = 0%.

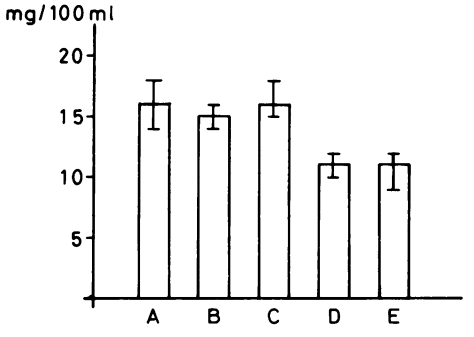

FIGURE 4. Lysozyme production of guinea pig lung macrophages 72 hr after incubation with electrocorundum and UICC asbestos at a dust concentration of 100 μg/10^6 cells: (*A*) control; (*B*) electrocorundum; (*C*) UICC chrysotile; (*D*) UICC crocidolite; (*E*) UICC amosite.

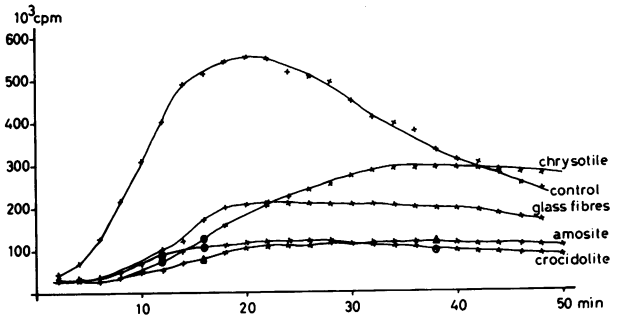

FIGURE 5. Phagocytosis determined by bioluminescence with opsonized zymosan following luminol enhancement 18 hr after incubation of guinea pig lung macrophages with asbestos and glass fibers. Graph shows one determination in counts per minute (cpm).

samples with a diameter of 3 μm are not toxic in our system.

A comparison of the results obtained when the cells were incubated in the presence or absence of serum gave no additional information on fiber toxicity. Glass fiber caused a considerably lower reduction in lactic acid production in comparison to the chrysotile and crocidolite fiber fractions with similar dimensions. Possibly, the surface properties of the different fibers play a greater role here than in causing the release of cytoplasmic enzymes. The influence of the UICC asbestos samples on lysozyme production was small and was only detected at high concentrations (Fig. 4). At very low concentrations we found an enhancement of cellular activity.

Figure 5 demonstrates the phagocytic activity of alveolar macrophages after 20 hr exposure to UICC asbestos samples and JM 100 glass fiber. Chemiluminescence generated by the phagocytic process was measured after Luminol enhancement. Using this assay it was shown that concentrations of 100 μg dust/mL caused a significant depression of phagocytosis for all four fiber types. Due to technical problems, only some of the results from this assay could be evaluated. In the future, we plan to investigate the effect of lower dust concentrations and longer exposure times on this test system in order to study the direct influence of dust loading on the phagocytic ability of the cells.

In conclusion, we wish to stress that more fiber fractions with narrowly defined length and diameter spectra are needed in order to make better and more meaningful comparisons between fibers with different chemical compositions.

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